# 4 INTRODUCTION TO THE MICROSCOPE

## 4.1 GOALS

4.1.1 To learn the theory and use of the various types of microscopes, e.g., stereo, compound, and phase contrast microscopes.

4.1.2 To learn the construction of various types of stereo and compound microscopes and the function of each component.

4.1.3 To obtain a working knowledge of factors determining the resolution of the microscope, including, but not limited to, total magnification and numerical aperture.

4.1.4 To learn proper care and maintenance of the equipment.

4.1.5 To learn proper achievement of Köehler illumination.

4.1.6 To learn the theory behind and the techniques for utilizing bright field and phase contrast microscopy.

## 4.2 TASKS

4.2.1 Apply proper alignment techniques necessary for phase contrast illumination when examining smears for spermatozoa. Refer to centering instructions for the microscope in use.

4.2.2 Apply proper techniques for obtaining Köehler illumination by examining spermatozoa on smears at various magnifications.

4.2.3 Perform bright field illumination techniques by examining spermatozoa on stained smears.

4.2.4 Perform phase contrast illumination techniques by examining stained and unstained smears for spermatozoa.

4.2.5 Perform routine maintenance on the equipment.

4.2.6 Read applicable literature. Refer to Appendix A and Appendix B.

## 4.3 TRAINING EVALUATION

4.3.1 Knowledge

4.3.1.1 Review of notes in training notebook by training coordinator.

4.3.1.2 Mini-mock trials/oral and practical examinations.

4.3.1.3 Completion of checklist by training coordinator.

4.3.2 Skills

4.3.2.1 Observation by training coordinator or designee.

4.3.2.2 Satisfactory performance on training exercises.

4.3.2.3 Completion of checklist by training coordinator.

4.4 RESOLVING POWER AND ILLUMINATION OF THE MICROSCOPE – TECHNICAL NOTES

4.4.1 Illumination

4.4.1.1 Good resolving power and optimum specimen contrast are prerequisites for good microscopy. Though the optics (ocular, objectives, and substage condenser) may be suitable, proper illumination is of paramount importance. The requirement for a good illumination system is uniform intensity over the entire field of view with independent control of light intensity, size of the illuminated field of view, and angular aperture of the illuminating cone.

4.4.1.2 Light intensity should be controlled for visual work by neutral density filters or by a variable voltage transformer on the light source.

4.4.1.3 A field diaphragm on the lamp housing usually controls the size of the illuminated field of view.

4.4.1.4 The angular aperture of the illumination cone is controlled with the substage iris.

4.4.2 Contrast and Resolution

4.4.2.1 For good contrast, the substage iris must usually be closed down slightly. This, however, cuts down the condenser aperture and decreases resolving power. It is necessary to operate with the substage iris open as far as possible, consistent with image contrast, to have good resolution.

4.4.3 Köehler Illumination

4.4.3.1 The best illumination for most purposes is a special type of critical illumination known as Köehler illumination (named after August Köehler, 1866-1948). Here, a specific secondary source is imaged in the specimen plane. The particular secondary source in this case is the uniformly illuminated lamp lens framed by the field diaphragm.

4.4.3.2 With Köehler illumination the imaging of the lamp lens and field diaphragm in the specimen plane yields three distinct advantages: 1) the ray paths are predictable and controllable; 2) the illumination is uniform; 3) the source size - that is, the area illuminated - can be adjusted.

4.5 PROCEDURE FOR KÖEHLER ILLUMINATION (Reference 1, Appendix B)

4.5.1 Determine that the lamp is centered according to the instructions for the microscope in use.

4.5.2 Using a medium to low power objective (approximately 10X), place a specimen in position and focus.

4.5.3 Close the field diaphragm.

4.5.4 Focus the image of the field diaphragm by adjusting the substage condenser.

4.5.5 Center the field diaphragm using the centering screws on the condenser.

4.5.6 Open the field diaphragm so that the rim just disappears beyond the field of view.

4.5.7 Adjust the condenser diaphragm (aperture diaphragm) to about ½ of the full aperture.

**NOTE:** Resolution, contrast, and depth of field can be regulated with the condenser diaphragm. It should not be used to regulate the brightness. For this purpose, either the regulating transformer or neutral density filters should be used.

INTRODUCTION TO THE MICROSCOPE STUDY QUESTIONS

1. Describe Köehler Illumination and how this is achieved on the microscope.
2. What is phase contrast microscopy?
3. What is bright field microscopy?
4. What are the major differences between the stereoscope, compound microscope, and phase contrast microscope?
5. What total magnifications are used when examining specimens under low and high power and how does one arrive at the total magnification?
6. What is an objective?
7. What is an eyepiece?
8. Briefly describe field diaphragm, aperture diaphragm, and substage condenser.
9. What are the major parts of the compound microscope?
10. What is resolution and resolving power and how is it determined?
11. Who is credited with developing the microscope?
12. What is a micrometer and how is it used?
13. What type of light source is used on microscopes?
14. What is refractive index and how does it affect microscopy?
15. What is numerical aperture?

CHECKLIST FOR INTRODUCTION TO THE MICROSCOPE

Name of Trainee:_____

1. Trainee has satisfactorily completed the following tasks:

   Applied proper alignment techniques necessary for phase contrast illumination while examining spermatozoa smears.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Applied the proper technique for obtaining Köehler illumination.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Performed bright field illumination techniques while examining spermatozoa on stained smears.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Performed phase contrast illumination techniques while examining spermatozoa on stained and unstained smears.

   Date:_____ Training Coordinator:_____

   Comments:_____

   Performed routine maintenance on the equipment.

   Date:_____ Training Coordinator:_____

   Comments:_____

2. Trainee understands the theory and use of the stereo, phase contrast, and compound microscopes, including the construction, components, and proper care of each.

   Date:_____ Training Coordinator:_____

   Comments:_____

3. Trainee exhibits a working knowledge of various resolution determining factors for the microscope.

   Date:_____ Training Coordinator:_____

Comments:_____

4. Trainee understands the theory behind and the practical application of the bright field and phase contrast microscopy techniques.

   Date:_____ Training Coordinator:_____

   Comments:_____

5. Notebook is organized and complete.

   Date:_____ Training Coordinator:_____

   Comments:_____

6. Trainee has participated in a mock trial and/or practical or oral examinations. Performance was satisfactory.

   Date:_____ Training Coordinator:_____

   Comments:_____

7. Trainee has read and understands all applicable literature.

   Date:_____ Training Coordinator:_____

   Comments:_____

♦END